Food Control 96 (2019) 299–309

Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

Gaseous chlorine dioxide maintained the sensory and nutritional quality of grape tomatoes and reduced populations of *Salmonella enterica* serovar Typhimurium

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ARTICLE INFO

Keywords:
Gaseous antimicrobial
Pathogen
Native microflora
Lycopene
Ascorbic acid
Texture
Nutrients

ABSTRACT

There have been numerous reports on the effectiveness of gaseous chlorine dioxide ($gClO_2$) in inactivating various human pathogens associated with fresh produce. However, studies dealing with both microbial reduction and impact on quality and nutrients of tomatoes are scarce. In the present study, gaseous chlorine dioxide was evaluated for its effectiveness on populations of *Salmonella* and native microorganisms on grape tomatoes, and impacts on sensory and nutritional quality. Grape tomatoes, dip inoculated with a cocktail of *Salmonella enterica* serovar Typhimurium, were treated with three different levels of $gClO_2$ generated by sachets containing sodium chlorite and reducing acids for up to 5 h in 20-L chambers. For the quality study, non-inoculated fruits were treated similarly with the $gClO_2$, and stored for 21 d at 10 °C. Sensory and nutritional quality, and native microflora were measured at 1, 7, 14 and 21 d of storage. Headspace $gClO_2$ concentrations and humidity in the chambers were measured during the treatments. Concentrations of $gClO_2$ in the chamber were relatively stable during the 2.5 and 5 h treatment times. Gaseous ClO_2 at 1.9 mg/L for 5 h and 4.3 mg/L for 2.5 and 5 h reduced *Salmonella* populations from 5.4 log CFU/fruit to a non-detectable level (detection limit 1.70 log CFU/fruit). However, populations of native microflora were not consistently affected by the $gClO_2$ treatments. Furthermore, the treatments did not have any significant effect on appearance, off-odor, firmness, color, or lycopene, and vitamin C contents of grape tomatoes during the 21-d storage. Overall, $gClO_2$ treatments that achieved more than 4 log reductions of *Salmonella* did not significantly ($P > 0.05$) affect sensory or nutritional quality of grape tomatoes.

1. Introduction

In recent years, the consumption of fresh and fresh-cut fruits and vegetables per capita has increased in the U.S. and around the world (Tapia & Welti-Chanes, 2012). Unfortunately, outbreaks of foodborne illness associated with consumption of fresh fruits and vegetables have also increased over the past decades (FDACS, 2006; Gabriela, Tomás-Callejas, Sbodio, Artés-Hernández, & Suslow, 2012; Trinetta, Vaidya, Linton, & Morgan, 2011). Although the etiological agents for more than 50% of outbreaks associated with fresh produce were unknown, in those cases which primary etiological agents were detected, bacteria, especially *Salmonella* spp. and pathogenic *Escherichia coli*, were often involved (Berger et al., 2010; Trinetta et al., 2011). Tomatoes have been known to be associated with outbreaks of foodborne illnesses. Approximately 90% of vine-stalk vegetable *Salmonella* outbreaks have been attributed to tomatoes (Jackson, Griffin, Cole, Walsh, & Chai, 2013). Grape tomatoes, more frequently consumed raw or used in salads than large tomatoes, have been found to be associated with *Salmonella* spp. As fresh produce is commonly consumed raw, presence of human pathogens increases the risk of outbreaks of foodborne illnesses and raises concerns from a public health perspective. Therefore, it is vital to develop treatments for the decontamination of raw tomatoes to reduce the potential for outbreaks of foodborne illnesses.

There is a lack of effective treatments for completely removing or inactivating these pathogens on fresh produce. It has been shown that aqueous sanitizers reduce, but not completely eliminate the number of microorganisms on the fruit, as microorganisms can infiltrate intercellular spaces, cracks, and crevices of produce (Tomas-Callejas et al., 2012). It is difficult for aqueous chemical sanitizers to penetrate all areas that pathogens may be lodged (Sy, Murray, Harrison, & Beuchat, 2005b). Therefore, it is unlikely that aqueous sanitizers can adequately inactivate foodborne pathogens on the fruit to meet microbiological

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https://doi.org/10.1016/j.foodcont.2018.09.023
Received 25 July 2018; Received in revised form 17 September 2018; Accepted 18 September 2018
Available online 21 September 2018
0956-7135/ Published by Elsevier Ltd.

quality goals.

Aqueous antimicrobials have a few disadvantages over gaseous sanitizers. First of all, wash water can be easily contaminated by pathogens during processing operations (Gabriela et al., 2012). As a result, there is a potential risk of pathogen contamination or cross-contamination of fresh produce in flume water and in dump tanks (FDACS, 2006). Accumulation of organic materials in the water systems can contribute to the cross-contamination for incoming fresh produce (Ongeng, Devlieghere, Debevere, Coosemans, & Ryckeboer, 2006). Using gaseous antimicrobials may avoid this type of cross-contamination. Secondly, microorganisms may become internalized during post-harvest handling of tomatoes, as some inappropriate washing procedures, such as negative temperature differentials between fruit and water, may allow contaminated water to infiltrate the interior of fruit (Bartz, 1983). In tomato handling, processing water temperatures of 40 °C or higher is often used to prevent water infiltration into tomatoes (Tomas-Callejas et al., 2012). Additionally, there is residual moisture present on the surface of fresh produce following aqueous chemical treatments, which can result in the growth of molds. Use of gaseous antimicrobials may avoid internalization of pathogens and minimize the growth of molds due to the presence of surface moisture in aqueous treatments. Finally, compared with aqueous antimicrobials, gaseous antimicrobials may have a greater ability to deliver sanitizers to the areas contaminated with pathogens such as stem scar areas of tomatoes (Sy et al., 2005b) where aqueous sanitizers cannot penetrate due to the surface tension.

Chlorine dioxide (ClO_2), being an entirely monomeric free radical, is a strong oxidizer (WHO, 2000) with 2.5 times the oxidizing capacity of chlorine in HOCl, and is widely used in paper manufacturing plants as a bleaching agent and in water treatment facilities as a disinfectant (Tzanavaras, Themelis, & Kika, 2007). Additionally, ClO_2 has less chance to form chloramines because of its inertness towards ammonia and organic materials.

Several factors can affect the performance of gaseous ClO_2 ($gClO_2$). Han, Floros, Linton, Nielsen, and Nelson (2001a) concluded that concentration, treatment time, relative humidity, temperature and pathogen location influenced the efficacy of $gClO_2$. Therefore, it is important to measure the actual ClO_2 concentrations during treatments as presence of organic matter in the treatment system can cause ClO_2 degradation. Large amounts of fresh produce samples in the treatment chamber would cause a more rapid degradation of ClO_2 than a small amount of sample (Yuk, Bartz, & Schneider, 2006).

Gaseous ClO_2 has been studied for its efficacy against human pathogens on a number of fruits and vegetables in past 2 decades. For example, *E. coli* O157: H7 on strawberries (Han, Linton, & Nelson, 2004), *Salmonella* and *Listeria* on hydroponic tomatoes (Bhagat, Mahmoud, & Linton, 2010), and *Salmonella* on raspberries, strawberries, and blueberries (Sy et al., 2005b). There are also numerous studies on quality of fresh produce as affected by $gClO_2$ (e.g. Gómez-López, Devlieghere, Ragaert & Debevere, 2007; Gómez-López, Ragaert, Jeyachchandran, Debevere, & Devlieghere, 2008; Sy et al., 2005a,b). However, almost all studies dealing with quality of fresh produce only measured simple quality parameters such as appearance, color and/or aroma (Gómez-López et al., 2007, 2008; Sy et al., 2005a,b). The only reports that addressed nutrients of fresh produce were from one single group of researchers (Vandekinderen et al., 2009a,b, 2008) who treated leeks, cabbage and carrots with $gClO_2$. However, the researchers only analyzed nutrients of the three products immediately after treatments (without additional storage) and found that $gClO_2$ reduced contents of vitamin C and carotenoids. Whether additional changes would occur during post-treatment storage is not evaluated. It is well known that fresh produce, being living organisms, undergoes changes in quality, particularly nutrients such as vitamin C, during post-processing (treatment) storage (Lee & Kader, 2000). Frequently, the changes in quality of fresh produce after intervention treatments can be observed only after additional storage (Mahajan, Caleb, Singh, Watkins, & Geyer, 2014). To the best of our knowledge, there is no report dealing with changes in nutrients of any produce item during post-$gClO_2$ treatment storage. In addition, there is no study of simultaneously evaluating both pathogen reduction and changes in sensory and nutritional quality of tomatoes. Therefore, in the present study, we evaluated the impact of $gClO_2$ on *Salmonella* populations, and sensory and nutritional quality of tomatoes during 21-d of post-treatment storage.

Tomatoes are a rich source of many health-benefiting phytochemicals, most noticeably ascorbic acid and red-colored carotenoid lycopene (Slimestad & Verheul, 2009; Veillet, Busch, & Savage, 2009). High levels of vitamin C and lycopene present in tomatoes and tomato products upon consummation can help to prevent oxidative damage (Halliwell, 2000), delay the pathogenesis of a variety of degenerative diseases, and prevent DNA mutation induced by oxidative stress (Lutsenko, Cárcamo, & Golde, 2002). Tomatoes are regarded as one of the most important sources of vitamin C in the human diet of many countries (Gahler, Otto, & Böhm, 2003). Lycopene not only contributes to the red color in tomatoes, but also reduces the risk of certain types of cancers (Demiray, Tulek, & Yilmaz, 2013). As a strong oxidant, ClO_2 could react with many phytochemicals such as ascorbic acid and lycopene (Napolitano, Green, Nicoson, & Margerum, 2005). One main cause of tomato lycopene degradation is oxidation (Shi & Maguer, 2000). However, there has been no report of $gClO_2$ impact on nutrients in tomatoes.

There have been numerous reports on the effectiveness of $gClO_2$ in inactivating various human pathogens associated with fresh produce, and a very limited number of studies on nutrients and the quality of fresh produce as affected by $gClO_2$. Often, studies focusing on nutrients often do not consider reductions of human pathogens associated with tomatoes. There have been no studies dealing with both the microbial reduction and impact on quality and nutrients related to $gClO_2$. Therefore, the objective of this study was to determine the efficacy of $gClO_2$ in inactivating *Salmonella*, native microflora, as well as yeasts and molds on grape tomatoes and the impact on fruit sensory and nutritional quality during 21 d of storage at 10 °C. Concentrations of $gClO_2$ and relative humidity as a function of time were also measured during the storage period.

2. Materials and methods

2.1. Source of tomatoes

Grape tomatoes were obtained from a farm in California through a major supermarket chain. The fruits were in transit for 5–7 d in refrigerated truck (4 °C). Upon receiving, the fruits were stored at 10 °C for up to 2 d before use in the study. The average weight of fruit was 10.2 ± 1.5 g. Before being treated with $gClO_2$, fruits were adjusted to ambient temperature (22 ± 1 °C) overnight. Only fruits without defects and blemishes were used in the study.

2.2. Effects on Salmonella populations

2.2.1. Bacterial cultures

Two strains of non-pathogenic *Salmonella enterica* serovar Typhimurium (ATCC 53647 and 53648) were used as surrogates in this study. Our earlier study demonstrated that these strains behaved similarly to a cocktail of five pathogenic *S. enterica* (Jiang et al., 2017). To minimize the influence of tomato native microflora, the strains were made nalidixic acid (Sigma-Aldrich, St. Louis, MO) resistant by successive growth of each strain in tryptic soy broth TSB (TSB, Difco, Becton Dickinson, Sparks, MD) with increasing nalidixic acid concentrations. Afterward, all strains were grown in TSB supplemented with nalidixic acid at a concentration of 100 ppm (100 μg/ml) at 37 °C for 24 h. Cultures were stored at 4 °C until use.